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Research Note

Genetic variability for cyanogen and trypsin inhibitor contents in sorghum (*Sorghum bicolor* (L.) Moench)

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Abstract
Sorghum is a staple food in the arid and semi-arid tropics, and is an important animal and bird feed in some developed countries. Very limited reports are available on anti-nutritional factors in sorghum. Grains of 200 sorghum genotypes including adapted cultivars and parental lines were characterized for the levels of cyanogens and trypsin inhibitor. Significant variation was observed for cyanogens (14.2-173.6 ppm) and trypsin inhibitor (1.6-12.8 TIU). The popular cultivars and parental lines had only moderate levels of these anti-nutritional factors. Selected 33 genotypes including the ones with extreme values were reanalyzed and the results were confirmed with high correlation for cyanogens ($r = 0.96$, $p < 0.001$), while in case of trypsin inhibitor a moderate correlation ($r = 0.41$, $p = 0.04$) was obtained. The results show that presence of cyanogens and trypsin inhibitors in sorghum do not pose a serious challenge to the nutritional quality as perceived previously in the developed world.

Keywords
Sorghum, cyanogens, anti-nutritional quality, trypsin inhibitor.

Sorghum is the fifth most important cereal crop by area (42.22 m ha) in the world, producing 62.30 m tonnes of grains (FAO, 2014), with Africa and Asia being the largest producers. Sorghum is an important staple crop in the arid and semi-arid tropics, acting as principal source of energy and other nutrients for millions of the poor people. Reports have shown that sorghum (Duodu *et al*., 2003) is inexpensive and nutritionally comparable or even superior to major cereals. The sorghum grains are rich in several phytochemicals and trace minerals because of which, these are now considered as "nutritious grains". As a staple food, sorghum is highly valued for human consumption and is used in many food preparations, the most common forms being boiled grains or ground flour. In developed countries like in the United States, Japan, Australia and in some developing countries like China, Mexico, etc. grains are important as animal and bird feed.

The perceived poor nutritional and processing quality of sorghum is because of presence of phytochemicals such as tannins, phytates, anthocyanins, phytosterols and policosanols (Awika and Rooney, 2004) and poor protein digestibility, which affects its use in foods and feed. Sorghums have a large number of bioactive compounds relevant to human health (Dykes and Rooney, 2006). Sorghum, especially the immature plant or fodder, contain cyanogenic glycoside, which liberates hydrogen cyanide upon enzymatic hydrolysis in the rumen and may be fatal to ruminants (Wheeler and Mulcahy, 1989; Cheeke, 1995). Cyanogenic glycosides account for approximately 90% of the wider group of plant toxins known as cyanogens. Cyanogens are anti-nutritional factors, the levels of which can vary widely with cultivar, plant parts or climatic conditions. Major cyanoglycoside present in sorghum leaves is Dhurrin (Simeonova and Fishbein, 2004). Potential toxicity arises from enzymatic degradation to produce hydrogen cyanide (HCN), resulting in acute cyanide poisoning. Trypsin inhibitors are another group of anti-nutritional factors in plant foods, which interfere with the enzymes trypsin and chymotrypsin. The trypsin inhibitors bind to these enzymes and make them non-functional and therefore protein digestion is impaired. The presence of trypsin inhibiting substances was shown in aqueous and acid extracts of sorghum grain powder (Filho, 1974). Studies on nutritional properties of sorghum grains are more or less limited to major entities like carbohydrates, protein and fat and very less reports are available on anti-nutritional factors like cyanogens and proteinase inhibitors. Therefore, an attempt was made to study the genetic variability for contents of cyanogens and trypsin inhibitors among 200 sorghum genotypes, and to ascertain whether these anti-nutritional factors render sorghum nutritionally inferior or not.

The materials for the study consisted of grain samples from popular sorghum cultivars and parental lines (49), advance breeding lines (34) and selected germplasm accessions (117) collected from major sorghum growing states. Replicated grain samples from each of the genotypes were subjected to the estimation of contents of cyanogen and trypsin inhibitor. Standard methodologies were adopted for the sample preparation and estimation as given below.

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Cyanogen: The estimation was based on the concept that cyanogenic glucosides on hydrolysis give equivalent (on molar basis) amount of HCN and p-hydroxybenzaldehyde (p-HB) (Gorz *et al*., 1977). p-HB in alkaline extract gives absorbance at 330 nm. 0.1 g of powdered sample was taken in a test tube and 10 ml distilled water was added and autoclaved for 30 min at 120^{o} C. After autoclaving, tubes were cooled immediately in cold water bath and an aliquot was removed and diluted with 0.1 N NaOH. The absorbance was measured at 330 nm and the values were expressed as ppm.

Trypsin inhibitor: The estimation was based on action of trypsin over BAPNA (benzoyl DL-arginine paranitroanilide hydrochloride), which results in p-nitroaniline (a colour compound). 0.1 ml Trysin (u/ml) + 0.5 ml sample extract (0.1 g/5 ml of water) was incubated at 37^{o}C. 0.2 ml BAPNA (0.5 mg/ml tris buffer of pH 8.2) was added and incubated for 1 h. Yellow colour developed was read at 410 nm. Trypsin activity without any sample extract was also read. The values were expressed as TIU (Hammerstrand *et al*., 1981).

Data analysis and confirmation: The replication-wise data were analyzed statistically using appropriate software and results were tabulated. Thirty-three genotypes, which showed either extreme values or important for different agronomic traits, were reanalyzed for both the factors to confirm the results. The Pearson correlation was used to determine relationship between the two sets of data as well as between the two anti-nutritional factors.

With growing interest on better health of the population through diet based interventions, like functional foods, it is necessary to detect any parameters in sorghum that would lead to impairment of its nutritional benefits compared to other fine cereals. Variations observed among the different category of genotypes for levels of cyanogen and trypsin inhibitor in the present study are described below:

Cyanogen: The analysis of variance indicated very high variation for cyanogens among the cultivars and parental lines as well as germplasm accessions (Table 1, Fig. 1). The widest range of 14.7-173.6 ppm was observed in germplasm accessions. The highest cyanogen content was in EA 10 (IC 345252) (173.6 ppm) collected from Tamil Nadu state, followed by GGUB 34 (IC 319877) (167.5ppm) collected from Madhya Pradesh. The advance breeding lines had lower levels of cyanogens, the lowest being 14.2 ppm in SPV 1762, a post-rainy season grain sorghum breeding line derived from cross involving landraces from Maharashtra.

Among the cultivars and parental lines, the lowest level of cyanogens was observed in AKR 354 (18.4 ppm) and 2219B (19.2 ppm). AKR 354 is the pollen parent of post-rainy season hybrid CSH 19R, while 2219B is the female parent of forage sorghum hybrid CSH 20MF and grain sorghum hybrids CSH 3 and CSH 6. Cyanogen is most important in case of forage sorghum and hence a lower level in 2219B is most desirable. The permissible/safe threshold limit for HCN in sorghum fodder is 200 ppm on dry weight basis and 500 ppm on fresh weight basis (McBee and Miller, 1980). However, very high levels of Cyanogen in the sorghum leaves, even up to 750-790 ppm, have been reported by Simeonova and Fishbein (2004). In another study, among 10 fodder sorghum varieties HCN contents ranged from 255 ppm (F-9601) to 347 ppm (Hegari), though the values were within safe limit of 500 ppm (Sarfraz *et al*., 2012). Most of the studies on cyanogens are confined to the fodder only and cyanogen content in the grains is not given much emphasis. However, Panasiuk and Bills (1984) reported that though the seeds of four cultivars of grain sorghum and four of sweet sorghum contained only traces (1 or 2 ppm) to 29 ppm of potential hydrocyanic acid (HCN) that could be generated as free HCN by digestion, the sprouts of the same cultivars grown for 3 days in the dark at 30°C contained 258 to 1030 ppm potential HCN relative to the weight of the ungerminated dry seed. Thus, lower level of cyanogen in the dry seeds is a preferred trait. Seeds of some of the fodder sorghum varieties in the present study, like HC 308 (37.6 ppm), Pant Chari 5 (34.7 ppm) and SSV 74 and SSV 84 (29 ppm) (both sweet sorghum varieties that are suitable for fodder purpose also) recorded low levels of cyanogen. However, a popular multi-cut forage sorghum variety, SSG 59-3 recorded higher level (100.4 ppm) of cyanogens.

When the 33 selected genotypes were reassessed for cyanogen levels, the mean values obtained were near to the previous results. The correlation between the two sets of values was ($r = 0.65$, $p < 0.01$) highly significant (Fig. 3) indicating the repeatability of the results. Excluding the values for two genotypes (EA 10 and SSG 59-3) for which very high deviation was observed, the correlation between old and new results raised to 0.96, indicating very high repeatability. Very high heritability (0.98-0.99) was also observed for this trait in all three classes of material studied (Table 1). Genotypes identified in the desirable direction for cyanogens that can act as potential donors in specific sorghum improvement programmes are listed in Table 2.

Trypsin inhibitor: Trypsin inhibitors are proteinase inhibitors that reduce the availability of biologically active trypsin. The range of trypsin

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inhibitor in all three groups of materials in the present study was nearly the same (Table 1, Fig. 2), but the germplasm accessions showed slightly lower mean value. The lowest level of trypsin inhibitory activity (1.6 TIU), was observed in EP 41 (IC 305922) and EP 38 (IC 305919) while high level of activity (12.2 TIU) was shown by ELG 25 (IC 568361) closely followed by SEVS 3 (IC 347569) (12.0 TIU). The germplasm collected from post-rainy sorghum growing regions of Maharashtra, Karnataka and Andhra Pradesh states (EP series) had in general lower levels of trypsin inhibitor with some exceptions. The distribution of proteinase inhibitors in sorghum cultivars and changes during germination was studied by Mulimani and Vadiraj (1991), who reported that chymotrypsin inhibitory activity was more pronounced than trypsin inhibitory activity in all sorghum varieties. On germination both trypsin and chymotrypsin inhibitory activities were completely reduced (Mulimani and Vadiraj, 1993). Upon dry heat treatment there was no appreciable change in proteinase inhibitory activity in sorghum, but moist heating reduced trypsin and chymotrypsin inhibitors to a greater degree. Significant reduction in trypsin inhibitory activities was also observed due to soaking, germination and fermentation (Osman and Gassem, 2013). In the present study, the trypsin inhibitory activities of the genotypes were found to be independent of seed coat colour, an indication that it is not correlated to tannin content. Similar observation was also reported by Rahman and Osman (2011).

Among the tested genotypes, only a few like RS 627, E 36-1, POP 52, SLV 19, RS 673, M 35-1 and 7B had higher levels of trypsin inhibitor activity (>10 TIU). Most of the popular cultivars had lower levels of trypsin inhibitor activity. Among the breeding lines majority had values in the range of low to medium (2-8 TIU). When the 33 samples were reanalyzed for trypsin inhibitor levels, the values were not highly repetitive ($r = 0.11$, $p = 0.53$) (Fig. 3), but exclusion of some outliers (SPV 1758, SPV 1762, POP 52, IS 2205 and CSV 17) improved the correlation ($r = 0.41$, $p = 0.04$). The nutritional significance of the enzyme inhibitors present in sorghum is not clearly understood and more research on enzyme inhibitors of cereal grains is needed. Some of the genotypes with lower levels of trypsin inhibitor are listed in Table 2.

Considerable variability for anti-nutritional factors such as cyanogen and trypsin inhibitor has been observed in the sorghum genotypes tested. The results could be ascertained with reanalysis of selected genotypes. Lower levels of these two factors in general indicate that presence of such anti-nutritional factors does not adversely affect the nutritional quality of sorghum. Nevertheless, presence of variability provides opportunity for genetic alteration through sorghum breeding programmes aimed at development of improved cultivars endowed with high yield potential along with desired levels of nutritional factors. Effect of processing needs to be studied, as techniques like heat treatment destroy protease inhibitors, but heat treatment also destroys some of the amino acids and vitamins. Therefore, standardization of processing techniques is essential for maintaining the nutritional value of sorghum.

References

Awika, J.M. and Rooney, L.W. 2004. Sorghum phytochemicals and their potential impact on human health. *Phytochemistry*, **65**: 1199-1221.

Cheeke, P.R. 1995. Endogenous toxins and mycotoxins in forage grasses and their effects on livestock. *J. Anim. Sci*., **73**: 909-918.

Duodu, K.G., Taylor, J.R.N., Belton, P.S. and Hamaker, B.R. 2003. Factors affecting sorghum protein digestibility. *J. Cereal Sci.*, **38**: 117-131.

Dykes, L. and Rooney, L.W. 2006. Sorghum and millet phenols and antioxidants. *J. Cereal Sci.*, **44**: 236-251.

FAO. 2014. Statistical database, Food and Agriculture Organization of the United Nations, Rome, Italy. (http://faostat.fao.org/) (Accessed on 1st August 2014)

Filho, J.X. 1974. Trypsin inhibitors in sorghum grain. *J. Food Sci.*, **39**: 422-423.

Gorz, H.J., Haag, W.L., Specht, J. and Haskins, F. 1977. Assay of p-hydroxybenzaldehyde as a measure of hydrocyanic acid potential in sorghums. *Crop Sci*., **17**: 578-582.

Hammerstrand, G.E., Black, L.T. and Glover, J.D. 1981. Trypsin inhibitor in soy products; modification of standard analytical procedures. *Cereal Chem*., **15**: 215-218.

McBee, G.G. and Miller, F.R. 1980. Hydrocyanic acid potential in several sorghum breeding line as affected by nitrogen fertilization and variable harvests. *Crop Sci*., **20**: 232-234.

Mulimani, V.H. and Vadiraj, S. 1991. Proteinase inhibitors of sorghum. *J. Sci. Food Agr*., **54**: 485-488.

Mulimani, V.H. and Vadiraj, S. 1993. Effects of heat treatment and germination on trypsin and chymotrypsin inhibitory activities in sorghum (*Sorghum bicolor* (L.) Moench) seeds. *Plant Food Hum Nutr*., **44**: 221-226.

Osman, M.A. and Gassem, M. 2013. Effects of domestic processing on trypsin inhibitor, phytic, acid, tannins and in-vitro protein digestibility of three sorghum varieties. *J. Agr. Tech*., **9**: 1187-1198.

Panasiuk, O. and Bills, D.D. 1984. Cyanide content of sorghum sprouts. *J. Food Sci.*, **49**: 791-793.

Rahman, I.E.A. and Osman, M.A.W. 2011. Effect of sorghum type (*Sorghum bicolor*) and traditional fermentation on tannins and phytic acid contents and trypsin inhibitor activity. *J. Food Agr & Env*., **9**: 163-166.

Sarfraz, M., Ahmad, N., Farooq, U., Ali, A. and Hussain, K. 2012. Evaluation of sorghum varieties/lines for hydrocyanic acid and crude protein contents. *J. Agr. Res. Pakistan*, **50**: 39-47.

 DOI: 10.5958/0975-928X.2016.00151.4

Simeonova, F.P. and Fishbein, L. 2004. Hydrogen cyanide and cyanides: Human health aspects. Concise International Chemical Assessment Document 61. World Health Organization, Geneva, Switzerland.

Wheeler, J.L. and Mulcahy, C. 1989. Consequences for animal production of cyanogenesis in sorghum forage and hay - a review. *Trop. Grassl*., **23**: 193.

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Table 1. Variation for anti-nutritional factors in sorghum genotypes

Category	Cyanogen (ppm)	Trypsin inhibitor (TIU/g)
Cultivars/Parental lines		
Mean	36.8	7.6
Minimum	18.4	2.7
Maximum	145.6	12.8
C.V. (%)	7.2	16.2
C.D. (0.05)	5.3	2.5
Heritability (h^2)(bs)	0.99	0.76
Breeding lines		
Mean	31.0	7.5
Minimum	14.2	1.9
Maximum	52.1	10.9
C.V. (%)	4.1	17.2
C.D. (0.05)	2.6	2.6
Heritability (h^2)(bs)	0.98	0.66
Germplasm accessions		
Mean	34.6	6.6
Minimum	14.7	1.5
Maximum	173.6	12.2
C.V. (%)	4.3	16.3
C.D. (0.05)	3.0	2.1
Heritability (h^2)(bs)	0.99	0.86

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Table 2. Sorghum genotypes identified with lower levels of anti-nutritional factors

Category	Genotype	Cyanogen (ppm)	Genotype	Trypsin inhibitor (TIU/g)
Cultivars / Parental lines	AKR 354	18.4	CSV 14R	2.7
	2219B	19.2	AKR 150	2.8
	2077B	19.9	SSV 84	3.3
	CSV 22	20.3	2219B	3.4
	CSV 19SS	22.6	2077B	3.5
	296B	22.9	AKR 354	3.5
	SLV 19	24.5	CSV 22	4.7
	IMS 9B	25.1	Urja	5.2
Breeding lines	SPV 1762	14.2	SPV 1759	1.9
	SPV 1758	18.2	SPV 1411	3.1
	SPV 1768	18.8	SPV 1755	5.1
	SPV 1802	20.2	SPV 1795	5.3
	SPV 1775	22.5	SPV 1756	5.6
	SPV 1764	23.3	SPV 1787	5.7
	SPV 1761	23.8	SPV 1760	6.2
	SPV 1757	24.5	SPV 1766	6.2
Germplasm accessions	SEVS 20	14.7	EP 41	1.5
	EP 97	17.0	EP 38	1.6
	PEC 7	19.0	EP 127	1.8
	PEC 15	19.5	EP 24	2.2
	IS 26866	19.7	EP 10	2.3
	EC 1	20.1	EP 48	2.6
	EP 16	20.6	EP 39	2.6
	EP 48	21.1	EP 22	2.7
	IS 18551	21.8	EP 21	2.7
	EP 115	22.1	EP 99	2.7
	PEC 5	22.9	EP 64	2.8
	EP 120	23.2	EP 57	2.8
	EP 99	23.2	EC 21	2.8
	EP 54	23.3	EP 107	2.8
	EP 105	23.4	EP14	2.9
	EP 17	24.6	EP 37	2.9

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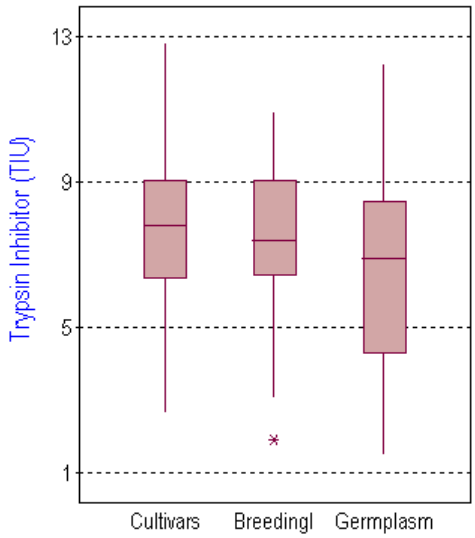

Figure 1. Variation for cyanogen content among sorghum genotypes

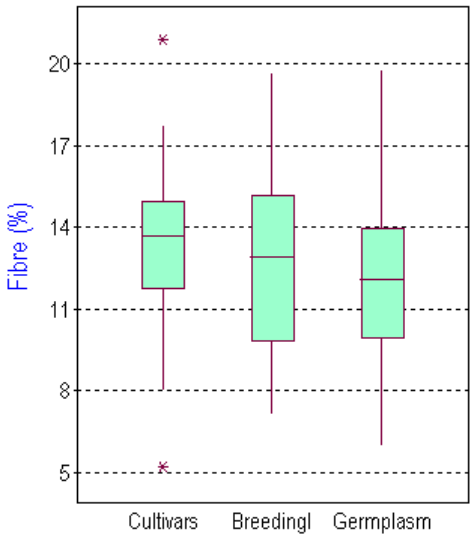

Figure 2. Variation for trypsin inhibitor content among sorghum genotypes

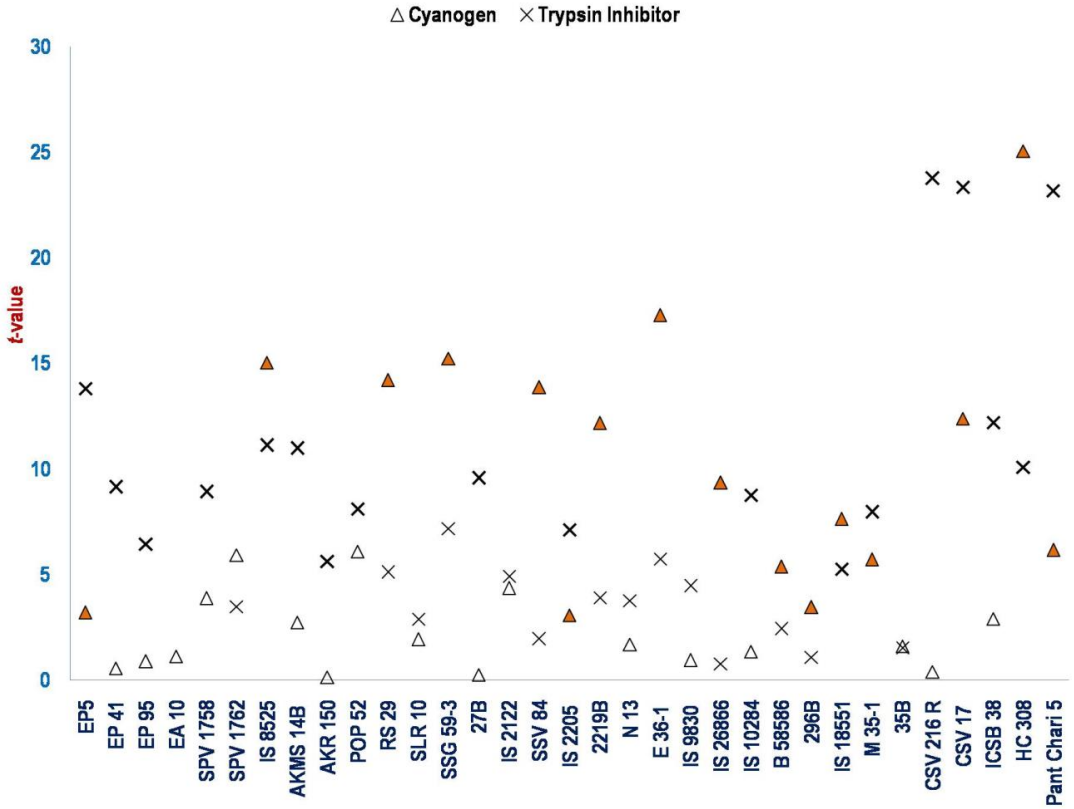

Figure 3. Plotting of student's *t*-value against the genotypes (solid fills indicate significant *t*-values)